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Photoinduced DNA Cleavage by Benzenediradical Equivalents: 1,3- and 1,4-Bis(dicarbonylcyclopentadienyliron)benzene

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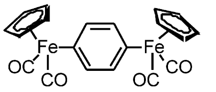
Abstract—Upon photolysis, diiron complexes 1,4- and 1,3-$Fp_2C_6H_4$ (**1** and **2**) linearize plasmid DNA at ratios as low as 1.5 and 3.0 molecules/bp DNA, respectively. Additionally, single-strand cleavage was observed at ratios higher than 0.05 and 0.19 molecules/bp DNA for **1** and **2**, respectively. Radical scavenging studies and metal radical control experiments implicate carbon-centered radicals as participants in the cleavage pathway.

The fundamental subunit for which the enediyne anticancer antibiotics are named is perfectly designed for double-stranded DNA cleavage because of its simultaneous production of two radical centers upon cycloaromatization, since the resulting benzenediyl can align in the minor groove to abstract hydrogen atoms from opposing strands of duplex DNA.[1] As a result, most studies aimed at producing arenediradicals have emulated this and similarly elegant rearrangements;[2] although others have taken the alternative approach of preparing 'diradical equivalents,'[3–5] aryl moieties functionalized with two independent single radical precursors. While benzenediyls have been produced and observed from such species under specialized conditions,[6] there are few reported examples employing two non-coupled single radical sources to cleave DNA; and none has apparently yielded true double-stranded DNA cleavage.[3,4]

We have previously shown that $CpM(CO)_nR$ complexes in which $M = W$ ($n = 3$) or Fe ($n = 2$) and $R = CH_3$ or C_6H_5 cause DNA cleavage via the photolytic generation of methyl or phenyl radicals.[7] While the tungsten complexes gave only single-strand cuts, both iron species yielded form III DNA, presumably arising from the accumulation of random, proximal single strand events. As a consequence, we anticipated that the rapid, successive photolytic generation of two radical centers on a single aromatic ring from the corresponding phenyldiiron species might lead to double strand cleavage. We now report the efficient formation of form III DNA by the photolysis of 1,4-$Fp_2C_6H_4$ (**1**) and 1,3-$Fp_2C_6H_4$ (**2**).



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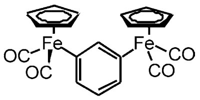

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Compounds **1** and **2** are both readily accessible by decarbonylation of the corresponding phenyldi(acyl-iron) species, which are prepared from the diacid chlorides and $NaFe(CO)_2Cp$.[8] The DNA cleaving activity of each of the complexes was determined using a plasmid relaxation assay (Fig. 1) to monitor the conversion of circular supercoiled DNA (form I) to relaxed circular (form II) and linear DNA (form III).

Each compound was photolyzed through a Pyrex filter in the presence of pBR322 DNA, and the amount of each form of DNA was assessed via agarose gel electrophoresis with ethidium bromide staining. Quantitation[10] of the bands in these gels indicated that form II DNA resulting from single strand cleavage was present at complex concentrations of 1.4 and 5.6 μM for **1** (a, lane 10) and **2** (b, lane 8), respectively.

Additionally, form III DNA was observed for the photolysis of **1** and **2** at 45 and 90 μM, respectively. These latter concentrations correspond to 1.5 and 3.0 mol-

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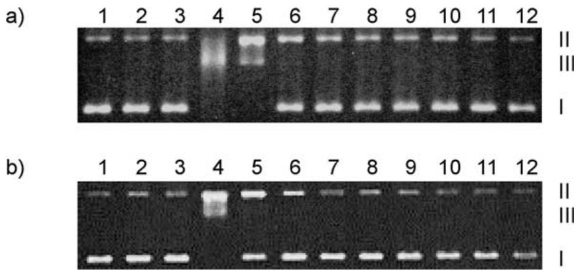


Figure 1. Photoinduced cleavage of pBR322 DNA (30 μM/bp in 10% DMSO/20 mM Tris buffer, pH 8) by **1** (a) and **2** (b). Lane 1, DNA alone; lane 2, DNA + complex (90 μM), no irradiation; lane 3, DNA alone, irradiated; lanes 4 through 12, DNA + complex (90, 45, 22.5, 11.3, 5.6, 2.8, 1.4, 0.7, and 0.35 μM, respectively). Mixtures in lanes 3–12 were irradiated with Pyrex-filtered light from a 450 W medium pressure mercury arc lamp for 20 min.[9]

ecules/bp, values similar to the ratio reported for the photoinduced cleavage of DNA by the naturally occurring enediyne dynemicin (0.75 molecules/bp[11]). However no reactions of either **1** or **2** with the plasmid yielded all three forms of DNA simultaneously, the hallmark of a correlated double-strand event.[12,13] Thus, the form III DNA obtained from the photolysis of **1** and **2** most likely results from the accumulation of proximal single strand breaks. Interestingly, neither of the diiron species was more efficient than the simple complex $CpFe(CO)_2C_6H_5$,[7] an outcome that is possibly due to the inabilities of **1** and **2** to align correctly in the minor groove of duplex DNA.

As with the monometallic complexes, the potential involvement of carbon-centered radicals in DNA cleavage by **1** was investigated by trapping studies (Fig. 2). Only one equivalent (vs **1**) of cysteine, a general radical trap,[14] was required to decrease the extent of strand scission (lane 5 vs lane 2); and 100 equivalents prevented nearly all cleavage (lane 3). Excess 4-hydroxy-TEMPO, a water soluble agent that scavenges carbon-[15] and metal-centered[16] radicals but not those based on oxygen, also reduced the amount of strand scission (lane 8 vs lane 7).

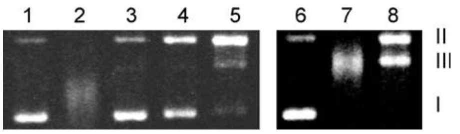


Figure 2. Effects of radical scavengers on the photoinduced cleavage of pBR322 DNA (30 μM/bp in 10% DMSO/20 mM Tris buffer, pH 8) by **1** (90 μM). Lane 1, DNA + complex, no irradiation; lane 2, DNA + complex; lanes 3–5, DNA + complex + cysteine (100, 10, and 1 equivalents vs metal complex, respectively); lane 6, DNA + complex, no irradiation; lane 7, DNA + complex; lane 8, DNA + complex + hydroxy TEMPO (100 equivalents). Mixtures in lanes 2–5, 7 and 8 were irradiated with Pyrex-filtered light from a 450 W medium pressure mercury arc lamp for 20 min.

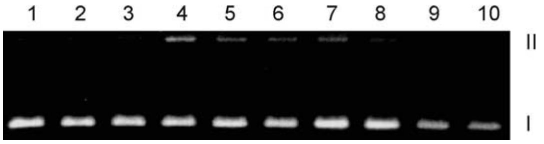
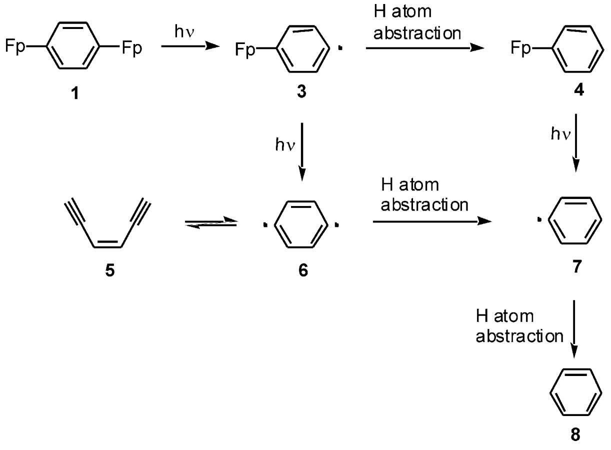


Figure 3. Relaxation of pBR322 DNA (30 μM/bp in 10% DMSO/20 mM Tris buffer, pH 8) by $[CpFe(CO)_2]_2$. Lanes 1 and 3, DNA alone; lanes 2 and 4–10, DNA + complex (90, 90, 45, 23, 11, 5.6, 2.8, and 1.4 μM). Reactions in lanes 3–10 were irradiated with Pyrex-filtered light from a 450 W medium pressure mercury arc lamp for 20 min.[7]

Any significant contribution of metal-centered radicals to cleavage was ruled out by generating $Cp(CO)_2Fe$ radical via the photolysis of the metal-metal bonded dimer $[Cp(CO)_2Fe]_2$[17] in the presence of the plasmid (Fig. 3). Only a minor amount of single-strand scission was observed at higher dimer concentrations, and no form III DNA was observed. The minimal activity exhibited by $[Cp(CO)_2Fe]_2$ probably does not involve hydrogen atom abstraction by the metal radical, since this process is disfavored both thermodynamically (as indicated by the relative bond dissociation energies of the metal hydrides[18] and hydrocarbons[19]) and kinetically.[20] Therefore, iron-based radicals are most likely not the primary active species leading to strand scission by either **1** or **2**. This conclusion is consistent with the previous implication of carbon radicals in DNA cleavage by photolysis of the monometallic systems $CpM(CO)_nR$.[7]

While the trapping studies are consistent with the participation of carbon-centered radicals in the cleavage process, these experiments cannot discriminate between production of the diradical **6** (Scheme 1) and the rapid successive formation/reaction of two independent radical centers, as in the conversion of **3** to **4** to **7**. The intermediacy of two radicals (whether simultaneously or sequentially produced) was indicated by the photolysis of **1** in THF-d_8, which gave rise to a peak with $m/z = 80$ (GC–MS), corresponding to a compound with the formula $C_6H_4D_2$. Importantly, this signal was not observed in experiments in non-deuterated solvents; and while consistent with the abstraction of two deuterium atoms from solvent, it does not rule out either mechanistic sequence. EPR experiments involving the photolysis of **1** in the presence of the spin-trap DMPO[21] were also inconclusive, giving rise to only a weak signal with hyperfine splittings $A_N = 15.5$ G and $A_H = 24.0$ G, values which fall within the reported range for other substituted and unsubstituted phenyl radical–DMPO adducts.[22] Unfortunately, 1,4-benzenediyl (**6**) itself is EPR silent, due to its singlet ground state;[23] and all



Scheme 1. General mechanism for the photolysis of **1**.

attempts to infer its intermediacy by producing its isomeric enediyne **5**[24] thermally or photochemically were unsuccessful. Therefore, on the basis of these experiments, it is impossible to determine whether the second radical is formed before or after the initial radical abstracts a hydrogen atom; and there is no kinetic or quantum yield data for the photolysis (for even the monometallic species $CpFe[CO]_2Ph$) in the chemical literature to address this issue.

In summary, the photolysis of the diiron phenyl complex **1** or **2** does produce linear form III DNA; however, this outcome appears to arise from the accumulation of single-strand damage. This result indicates either that the diradical is not formed or that it is not positioned appropriately to abstract hydrogen atoms from opposing strands of duplex DNA.

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